Biomarker-Based Prediction of Tyrosine Kinase Inhibitor Cardiotoxicity using Human iPSC-Derived Cardiomyocytes

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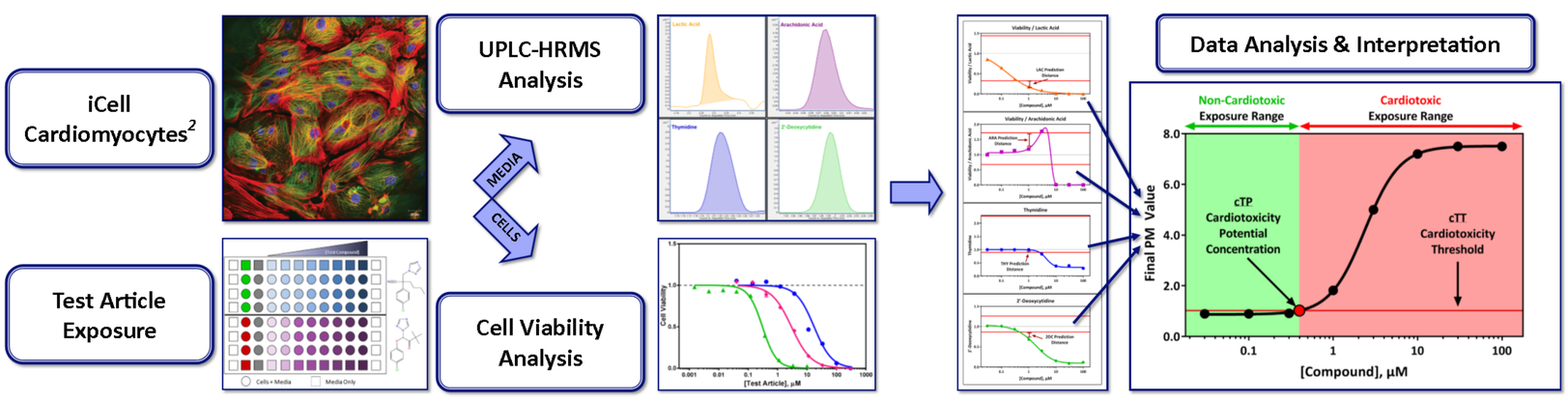
ABSTRACT

- Tyrosine kinase inhibitors (TKI) have greatly improved the treatment and prognosis for a wide range of cancers. Unfortunately, numerous TKIs produce cardiotoxic effects, which were not well predicted during preclinical studies.
- We developed an *in vitro* assay, Cardio *quick*Predict, for predicting cardiovascular liability based on changes in human iPSC-derived cardiomyocytes (iPSC-CM) metabolism and cell viability, which identifies both functional and structural cardiotoxicants. The assay's prediction model (PM) is based on the response of four metabolites (lactic acid (LAC), arachidonic acid (ARA), thymidine (THY), and 2'-deoxycytidine (2DC)) and predicts the concentration at which a drug exhibits cardiotoxicity potential. The PM classified 81 drugs with known cardiotoxicity outcomes (54 cardiotoxic, 29 non-cardiotoxic) with 86% balanced accuracy, 83% sensitivity, and 90% specificity.
- The current study evaluated the utility of this assay for evaluating the cardiotoxicity potential of TKIs. We tested 10 TKIs that induce a variety of cardiotoxic effects, including eight drugs clinically associated with cardiotoxicity (crizotinib, dasatinib, imatinib, lapatinib, nilotinib, sorafenib, sunitinib, and vandetanib) and two drugs considered to be relatively cardiac-safe (axitinib and erlotinib) to compare changes in metabolism of the PM ratios. Human iPSC-CMs were exposed to eight concentrations of each drug for 72 hours and cell viability and metabolites in the spent media were analyzed.
- Every drug altered at least one metabolite independent of a change in cell viability. Crizotinib, imatinib, sorafenib, sunitinib, and vandetanib elicited a response in all four metabolites indicative of cardiotoxicity; however, a difference was observed in which metabolite was impacted at the lowest concentration. For example, crizotinib altered LAC at significantly lower concentrations (≥5-fold) than where a response was observed in ARA and THY. In contrast, sorafenib elicited a response in ARA prior to LAC, THY, and 2DC.

METHODS

Cardio quickPredict *Workflow*



Cardio *quick*Predict: Exposure-Based Prediction of Cardiotoxicity

* The PM is based on the response of four metabolites (alone or normalized to viability): lactic acid (LAC), arachidonic acid (ARA), thymidine (THY), and 2'-deoxycytidine (2DC).
* Metabolite-specific thresholds were previously set with diverse training set of drugs and optimized for balanced accuracy. These thresholds are used to calculate the prediction distance for each metabolite based on the difference between the threshold and the hiPSC-CM response at a given concentration.
* The final PM value is the maximum prediction distance at each concentration. These values are used to determine the concentration where a drug alters hiPSC-CM metabolism at a level indicative of cardiotoxicity potential.
* The Cardiotoxicity Potential Concentration (cTP) is predicted based on the point where the concentration-response curve for the final PM values crosses the Cardiotoxicity Threshold (cTT).

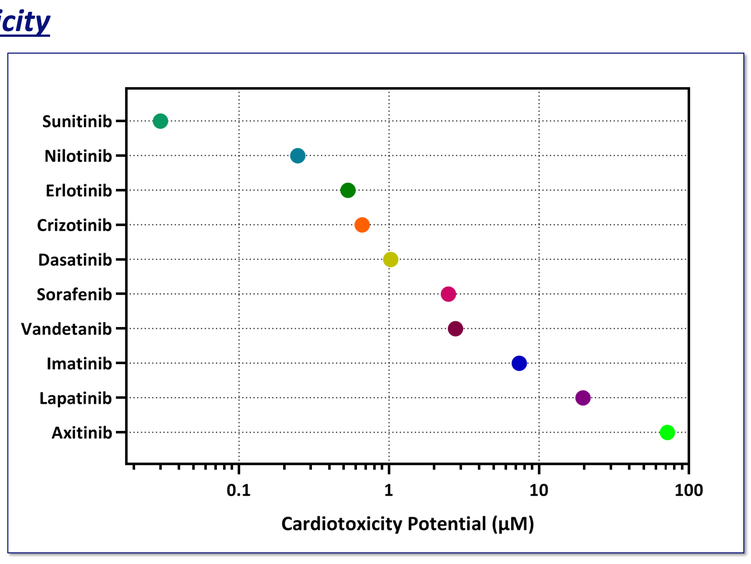
- Human induced pluripotent stem cell-derived cardiomyocytes (hiPSC-CM, iCell Cardiomyocytes², FUJIFILM Cellular Dynamics, Inc.) were plated in 96-well plates and exposed to drug for 72 hours.
- Spent media from the last 24-hour treatment period was collected for UPLC-HRMS analysis and cell viability was assessed with the CellTiter-Fluor Cell Viability Assay (Promega).
- Samples were analyzed with rapid UPLC-HRMS method optimized for the predictive metabolites .
- Each metabolite was normalized to the reference treatment samples (0.1% DMSO). Non-linear concentration-response curves were fit for each endpoint using GraphPad Prism.
- The prediction distance for each metabolite in the Cardio *quick*Predict PM was calculated at each concentration and the final PM value was determined.
- The final PM values were fit with a non-linear concentration-response curve and the "Cardiotoxicity Potential Concentration" (cTP) was predicted based on the point where the concentration response curve crossed the "Cardiotoxicity Threshold".

RESULTS

TKI-Induced Changes in hiPSC-CM Metabolism Indicate Potential for Cardiotoxicity

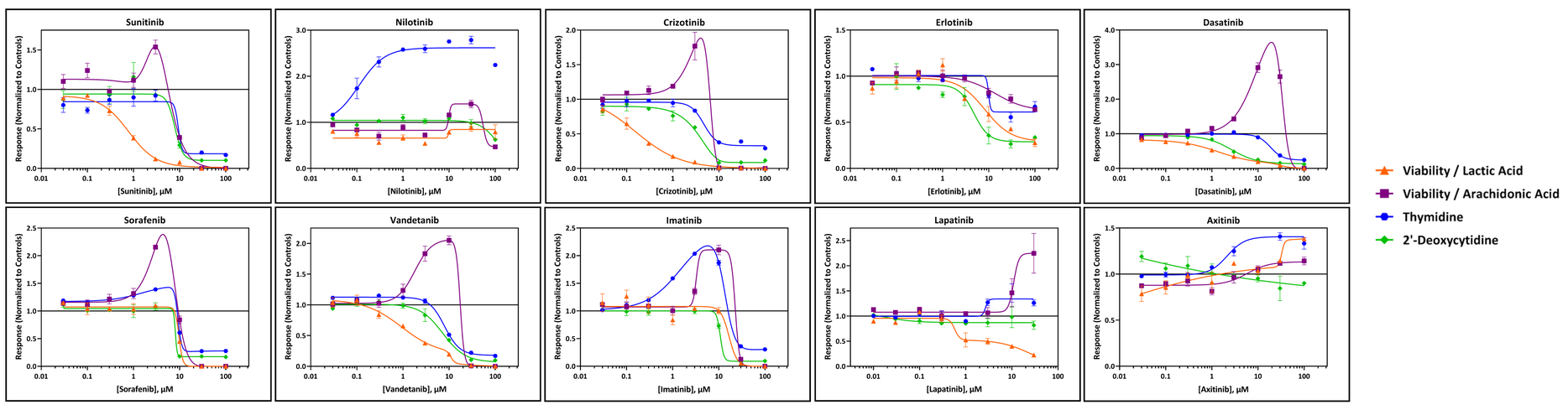
Compound	Target	CVS Adverse Event in FDA Label[1]	Total C_{max} (µM)	cTP (µM)	Safety Margin[2]
Sunitinib	VEGFR1/2/3, c-Kit, PDGFR, FLT3, RET, CSF1R	Myocardial ischemia, myocardial infarction, LVEF decrease, congestive heart failure, prolonged QT intervals and torsade de pointes, hypertension	0.25	0.03	0.12
Nilotinib	Bcr-Abl, c-Kit, PDGFR	QT prolongation, Sudden deaths, vascular occlusive events	4.2	0.2	0.08
Crizotinib	c-Met, ALK	QT prolongation, bradycardia	3.0	0.7	0.14
Erlotinib	EGFR	Myocardial infarction (rare); "Cardiac-Safe"	3.9	0.5	0.91
Dasatinib	Bcr-Abl, c-Kit, PDGFR, Src	Cardiac dysfunction, pulmonary arterial hypertension, QT prolongation	3.8	1.0	1.43
Sorafenib	VEGFR2/3, c-kit, PDGFR, FLT3, RAF1, BRAF	Cardiac ischemia and/or infarction, hypertension, QT prolongation, LVEF decrease, congestive heart failure	0.72	2.5	0.15
Vandetanib	VEGFR2, EGFR, PDGFR, RET	QT prolongation, torsades de pointes, sudden death, heart failure, hypertension	0.73	2.8	0.84
Imatinib	Bcr-Abl, c-Kit, PDGFR	Severe congestive heart failure, LV dysfunction, cardiogenic shock	16.6	7.4	1.89
Lapatinib	EGFR, ERBB2	LVEF decrease, QT prolongation	3.3	19.7	4.70
Axitinib	VEGFR1/2/3, c-Kit, PDGFR	Hypertension, cardiac failure (rare); "Cardiac-Safe"	0.07	71.1	1024.47

[1]Summarized from Yang and Papoian, J Appl Toxicol. 2018; 38(6):790-800; [2]Safety Margin = cTP/Total C_{max}



- The cTP concentration (determined from the final PM values) can be used to identify which drugs may lead to cardiotoxicity at lower exposures.
- If known, the therapeutic C_{max} can be compared to the cTP concentration to understand the safety margin for the drug and if the observed effect occurs at therapeutically relevant concentrations.
- Using a safety margin of 10 (cTP < 10×C_{max}), the assay correctly predicted the cardiotoxicity potential for 9/10 TKI (erlotinib is predicted to be cardiotoxic).

Metabolites Impacted Differently by TKI Indicating Multiple Mechanisms of Cardiotoxicity



- LAC, ARA, THY, and 2DC have key roles in mitochondrial function and replication as well as modulating oxidative stress. The effects observed following TKI exposure are indicative of potential mechanisms of cardiotoxicity. For example:
  - ♦ Altered hiPSC-CM LAC secretion provides insight into the state of hiPSC-CM mitochondrial energy metabolism and is reflective of the cell's decreased ability to produce ATP via oxidative phosphorylation.
  - ♦ Drug-induced dysregulation of THY or 2DC indicates that the drug may interfere with mitochondrial DNA synthesis and replication.
  - ♦ Changes in arachidonic acid metabolism, which may reflect dysregulation of lipid transport or metabolism, fatty acid metabolism, or a redox state unbalance.

CONCLUSIONS

- The Cardio *quick*Predict assay accurately identified the cardiotoxicity potential for 9 of the 10 TKI evaluated in this study.
- TKI impacted the four metabolites included in the PM at different concentrations. These differences can be used to help understand a drug's mechanism of toxicity.
- These results suggest that the Cardio *quick*Predict assay can be used to screen for cardiotoxicity potential of TKIs and can identify TKIs that cause a range of functional and structural cardiotoxic effects.
- The metabolic readout of this assay can be combined with other assays, such as the CiPA myocyte assay, to provide a more comprehensive evaluation of a drug's cardiovascular liability and greater mechanistic insight.

Acknowledgments

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For more information on the Cardio *quick*Predict assay and a copy of this poster, please contact Jessica Palmer at jpalmer@stemina.com.